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Technical Explanation

US Application 10/583,706

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Claim 1 of the present application

(TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

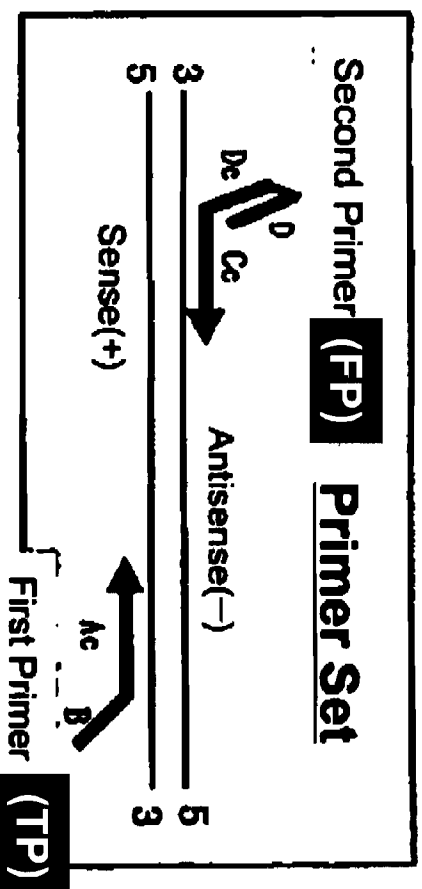
a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

*The first primer is **TP**, the second primer is **FP**.

TP; Turn-back Primer

FP; Folded Primer

Technical explanation of the TP and FP

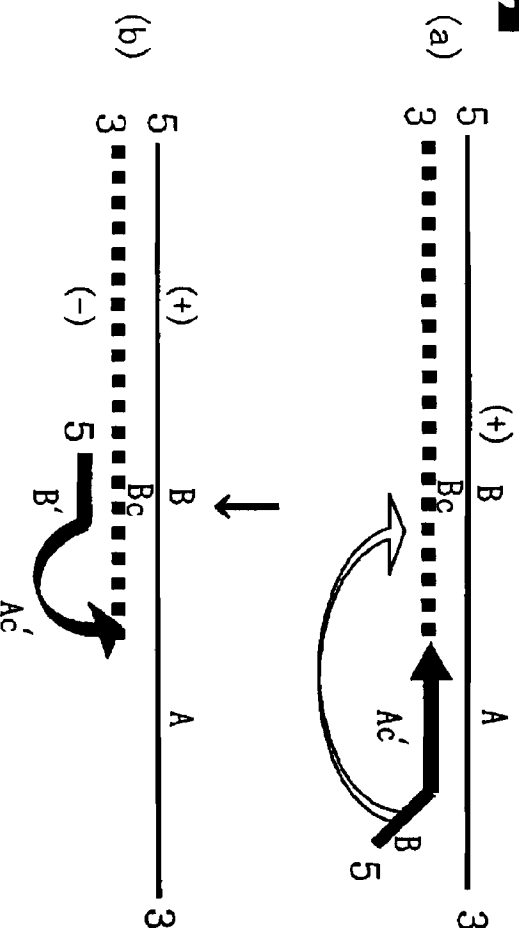


FP has the function as follows;

- (1) FP has the folded sequence (D- (a) Dc') in the 5' side sequence.
- (2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.
- (3) The folded sequence (D-Dc') **DO NOT** hybridize to the elongation strand from FP.

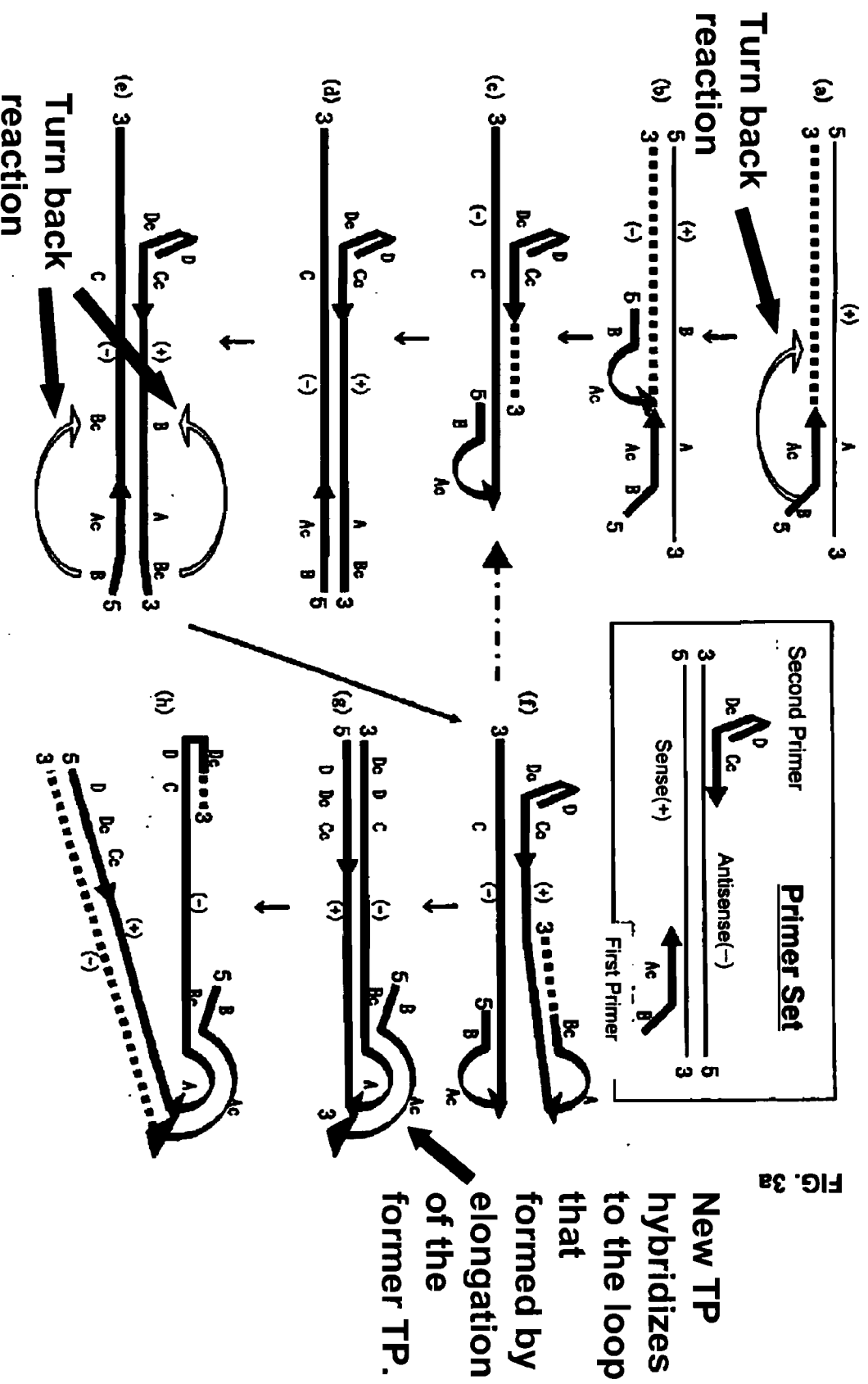
TP has the function as follows;

- (1) TP has the turn back portion (B) in the 5' side sequence.
- (2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.



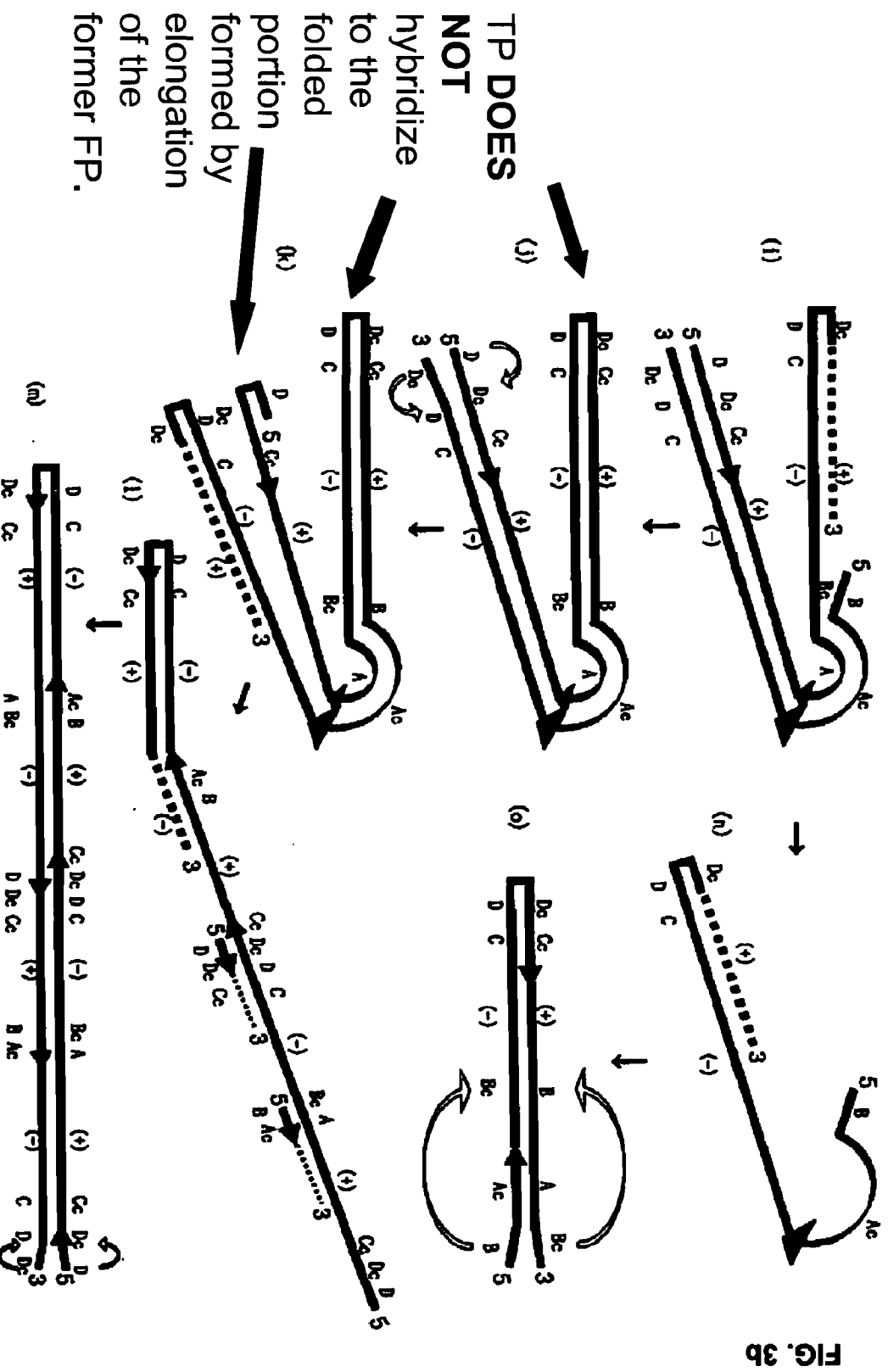
Mechanism of the amplification reaction of the TP-FP(1)

(FIG.3 of the present invention)



Mechanism of the amplification reaction of the TP-FP(2)

(FIG.3 of the present invention)



The present invention has four advantages.

(1) Isothermal amplification

- The amplification occurs without thermal denaturation.

(2) Specific amplification

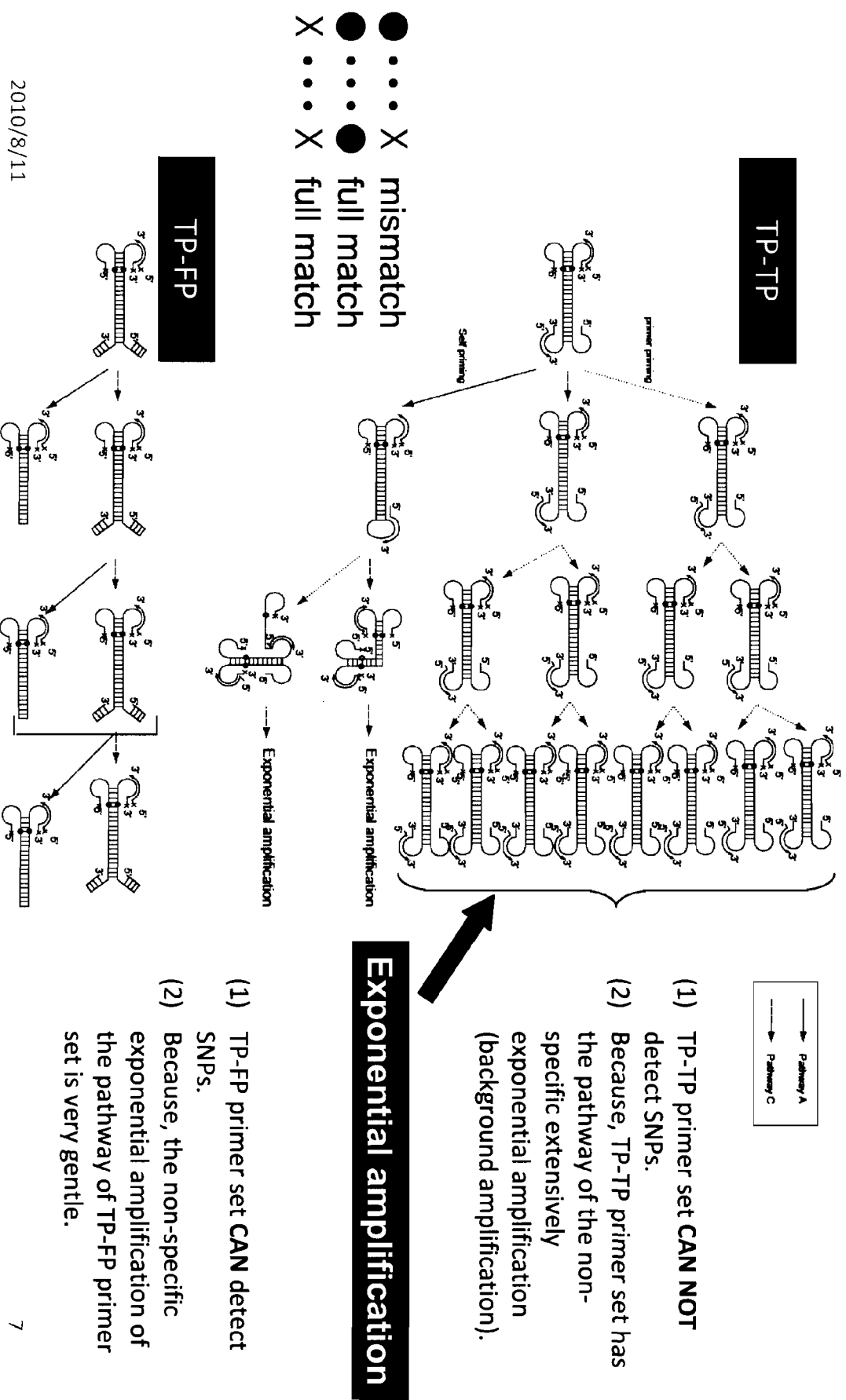
- The present invention can detect SNPs without non-specific amplification.

(3) Short time amplification

(4) Easy primer design

Mechanism of specific amplification

The non-specific amplification **DOES NOT** occur in the present invention (TP-FP).



Short time amplification and easy primer design(1)

(1) TP

- (i) TP can amplify exponentially.
- (ii) TP has a strong engine of amplification.
- (iii) TP has two area depending on template sequence.

(2) FP

- (i) FP can not amplify exponentially, but amplify linearly.
- (ii) FP is like a mirror which reflect TP amplification.
- (iii) FP needs only one area depending on template sequence.

(3) TP-TP primer set

- (i) TP-TP primer set needs four areas depending on the template sequence.
- (ii) TP-TP Primer set needs a design of a couple of good TP because the reaction is totally controlled by no good TP.
- (iii) TP-TP Primer set is difficult to design.

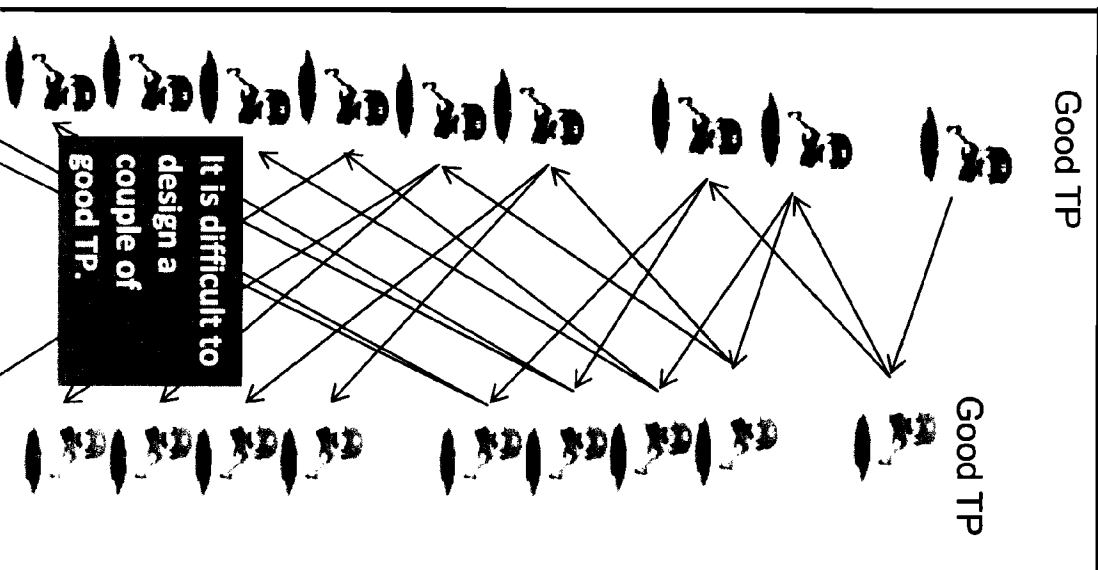
(4) TP-FP primer set

- (i) TP-FP primer set needs only three areas depending on the template sequence.
- (ii) TP-FP Primer set needs a design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.
- (iii) TP-FP Primer set is easy to design.

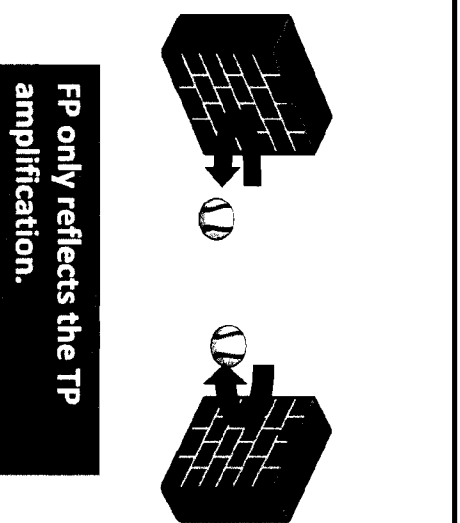
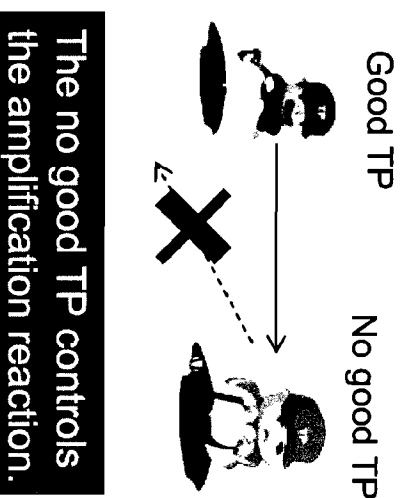
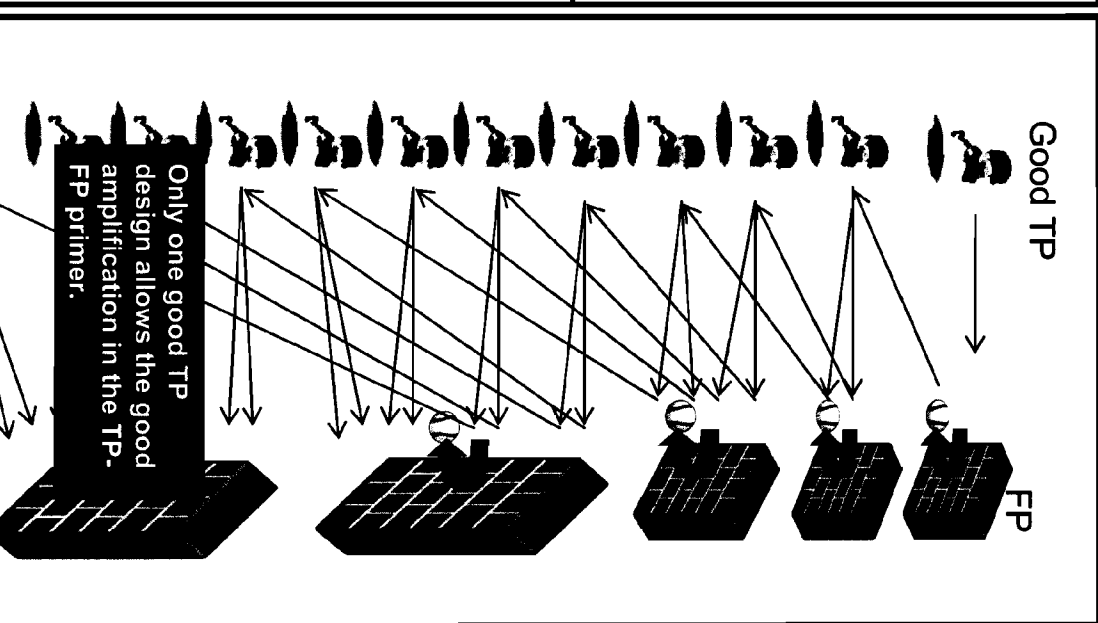
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Short time amplification and easy primer design(2)

TP+TP



TP+FP



Office Action (1)

Summary of the Office Action

The examiner pointed out as follows;

- (1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below left) in Rabbani (EP0971039A2).
- (2) FP are shown in Figure 1, step 3 (③ shown in below right) in Rabbani.
- (3) Therefore, Claims 1 to 5 of the present invention lacks novelty (102(b)).

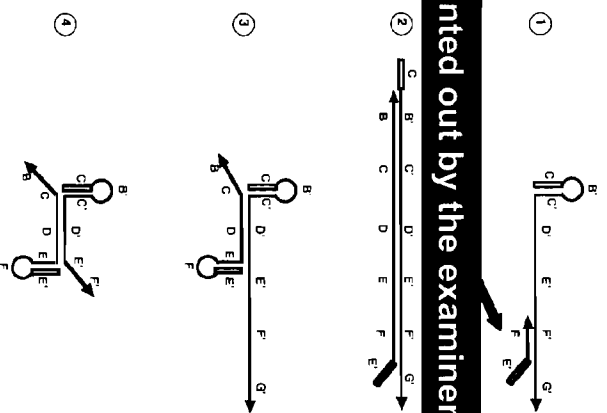


FIGURE 4

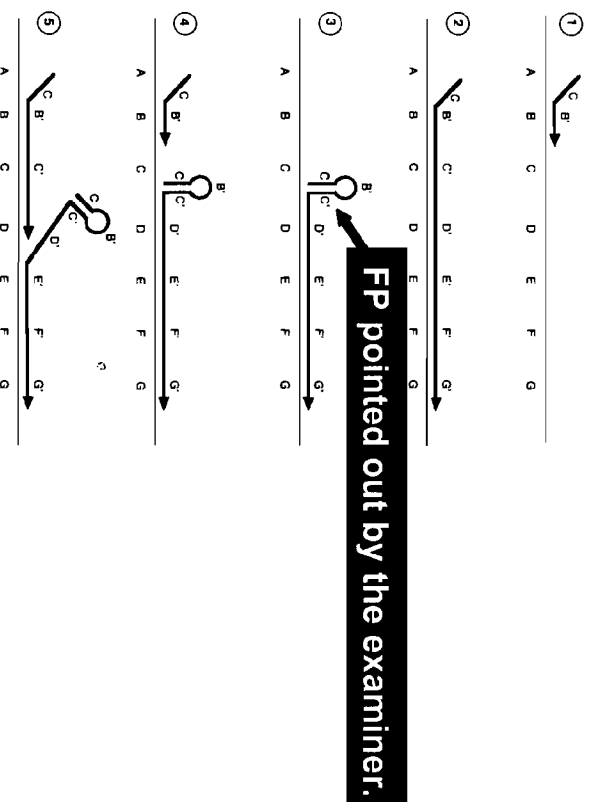


FIGURE 1

Office Action (2); FIGURE 1 ③ in Rabbani is NOT FP.

- (1) FIGURE 1 ③ in Rabbani shows the elongation strand from TP.
- (2) **Primer is different from the elongation strand.**
- (3) Rabbani **DOES NOT** show the TP-FP primer set of the present invention.

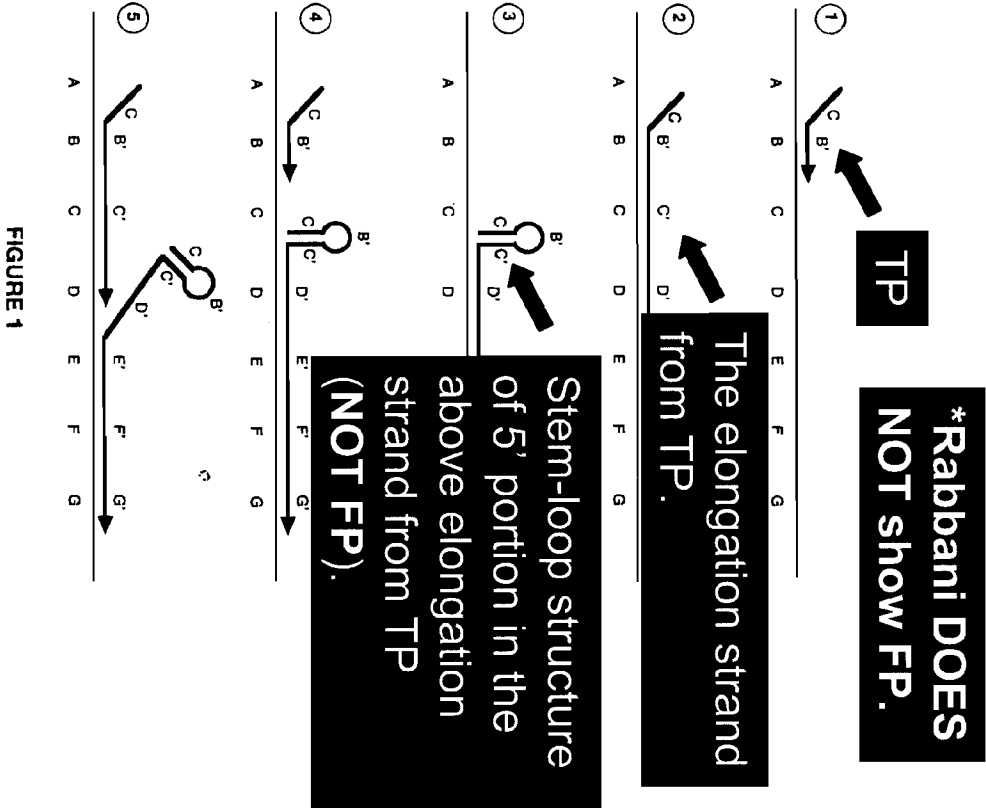
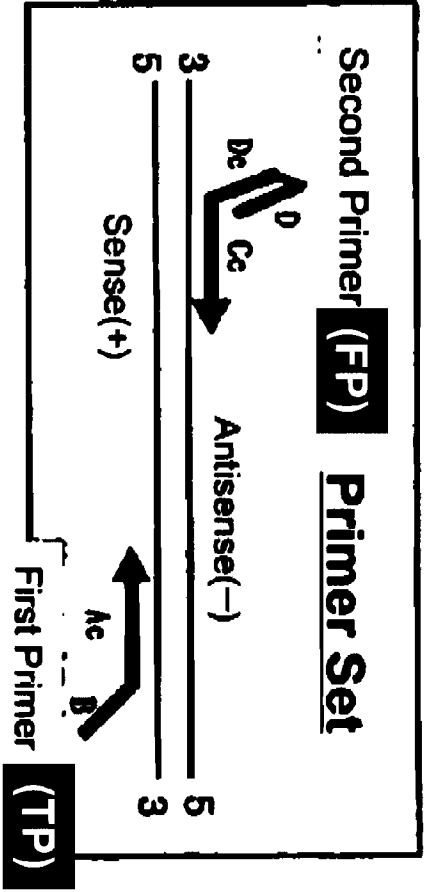
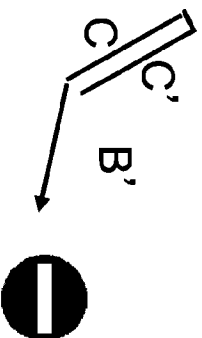
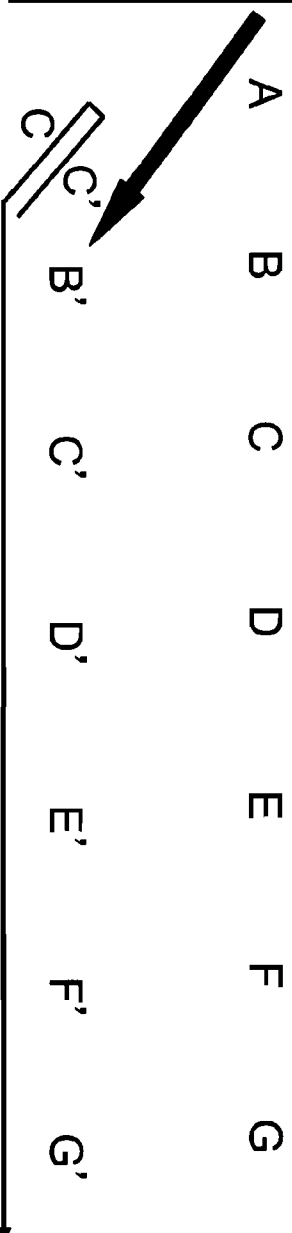


FIGURE 1

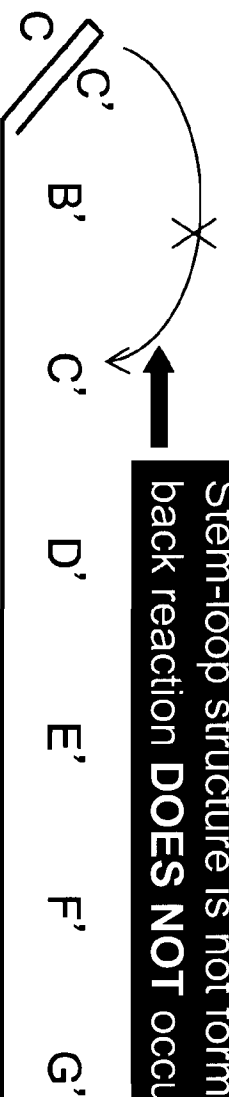
In case FP is used in the FIGURE 1 in Rabbani.



The sequence B' **DOES NOT** form stem-loop structure (B **DOES NOT** form a single strand).



New primer **DOES NOT** hybridizes to the sequence B in the template nucleic acid.



Stem-loop structure is not formed (Turn back reaction **DOES NOT** occur).

